1: Biochemistry 1993 Jun 15;32(23):6116-20 Related Articles, NEW Books

Rapid kinetic analysis of a point mutant of HIV-1 reverse transcriptase lacking ribonuclease H activity.

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The comparative kinetics of RNA-dependent DNA polymerization catalyzed by wild-type HIV-1 reverse transcriptase and a point mutant specifically lacking RNase H activity were analyzed using a heteropolymeric substrate consisting of a 19-mer primer hybridized to a 345-nucleotide RNA template. The rapid-quench product distributions generated under single-turnover conditions, in which primer extension by the two enzymes was restricted to the incorporation of 5 nucleotides (N+5), were significantly different. Whereas the wild-type enzyme catalyzed synthesis of the N+5 product over the time course of the reaction (20 ms-10 s) with a relatively low degree of processivity, the extent of accumulation of the intermediate N+2 and N+3 products was grossly exaggerated in the parallel mutant-catalyzed time course. The observation of concomitant polymerase-dependent hydrolysis during the course of synthesis catalyzed by the wild-type enzyme suggested that the inability of the RNase H- mutant to hydrolyze the RNA template created blocks to further synthesis by reducing the rates of DNA polymerization at these intermediate positions, and hence impaired the ability of this mutant to complete cDNA synthesis.

PMID: 7685188 [PubMed - indexed for MEDLINE]

1: Mol Biotechnol 1997 Aug;8(1):61-77

Reverse transcriptase. The use of cloned Moloney murine leukemia virus reverse transcriptase to synthesize DNA from RNA.

QH506 .M65

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Reverse transcriptase (RT) is the key enzyme required for conversion of RNA to DNA. Cloning of Moloney murine leukemia virus (MMLV) RT has enable engineering an RT that lacks endogenous RNase H activity. RT catalyzes cDNA synthesis more efficiently in the absence of RNase H. We describe here a number of properties of MMLV RT and RNase H-minus MMLV RT not summarized in a single location elsewhere, providing a basis for best use of these enzymes in cDNA synthesis. In addition, general guidelines and detailed protocols are provided for use of MMLV RTs in one tube double-stranded cDNA synthesis, in [32P]cDNA synthesis, and in RT-PCR and long RT-PCR.

PMID: 9327398 [PubMed - indexed for MEDLINE]

1: Biotechniques 1997 Jun;22(6):1119-22, 1124-6

Analysis of the size distribution of first-strand cDNA molecules.

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We have developed a method to analyze the size distribution of the first-strand cDNA molecules corresponding to given mRNA species. First-strand molecules synthesized from cytoplasmic polyadenylated RNAs are separated by electrophoresis on an alkaline agarose gel, and a Southern blot hybridization is performed. As an example, we analyzed the first-strand molecules corresponding to the human c-myc mRNAs. This method can be used to determine whether full-length, first-strand molecules corresponding to an mRNA species to be cloned are synthesized efficiently. Interestingly, this method allows one to analyze full-length, first-strand cDNA molecules with a much higher resolution than Northern blot analysis of mRNA molecules. This method can therefore be used to discriminate between the multiple mRNA species transcribed from a given gene or the homologous mRNA species transcribed from a given gene family.

PMID: 9187762 [PubMed - indexed for MEDLINE]

Reverse transcriptase and its associated ribonuclease H: interplay of two enzyme activities controls the yield of single-stranded complementary deoxyribonucleic acid.

Berger SL, Wallace DM, Puskas RS, Eschenfeldt WH.

The synthesis of single-stranded globin cDNA by the RNA-directed DNA polymerase activity of reverse transcriptase in the presence of oligothymidylate primers was investigated in order to determine the limitations to higher yields. The results indicated that the associated ribonuclease H activity, an integral part of reverse transcriptase, plays a large role in the synthesis of the first strand of cDNA and that the interplay of the two enzyme activities for any specific set of conditions determines the yield of single-stranded products. In both the presence and the absence of polymerization, the associated ribonuclease H catalyzed the deadenylation of mRNA, producing molecules that were somewhat shorter, highly homogeneous in size, and fully translatable into globin protein. They were also entirely lacking in the ability to serve as templates for cDNA synthesis. The reaction was completely dependent on oligothymidylate and completely independent of deoxyribonucleoside triphosphates. The initial rate of deadenylation was one-fourth the initial rate of initiation of polymerization when saturating levels of deoxyribonucleoside triphosphates were used in the polymerase reaction. In the presence of ribonuclease H activity, the DNA polymerase catalyzed the synthesis of an array of cDNAs including some that were full length. The initiation of polymerization was rate limiting: once synthesis had begun, it required 1-1.5 min to transcribe globin mRNA. However, most primers that were elongated were aborted prematurely. Maximum synthesis of full-length cDNA required stoichiometric levels of enzyme and high triphosphate levels, but regardless of conditions, the sum of completed cDNA and deadenylated mRNA accounted for only 50% of the input mRNA. The data fit a model in which synthesis of full-length cDNA molecules depends on the arrangement of primers and transcription initiation complexes on the poly(A) "tail" of mRNA.

PMID: 6190507 [PubMed - indexed for MEDLINE]

1: Proc R Soc Lond B Biol Sci 1991 Mar 22;243(1308):235-9

Reverse-transcriptase-associated RNaseH activity mediates template switching during reverse transcription in vitro.

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During the first steps of reverse transcription of the retroviral genome, sequences present at the extremities of the RNA are used to reconstitute a host cell PolII promoter. The assembly of the promoter occurs by template switching, which takes advantage of a direct repeat at the ends of the RNA molecule. These steps are catalysed by the viral reverse transcriptase, which carries an intrinsic RNaseH activity that is probably also involved therein. To study the role of the RNaseH activity in this first template-switching event, an in vitro system has been developed based on primer extensions of synthetic RNAs. When an RNA was reverse transcribed with wild-type reverse transcriptase in the presence of a second RNA the 3' part of which was repeated at the 5' end of the first one, extension products could be observed corresponding to a chimeric cDNA comprising both RNA species. This template switching could not be detected when a mutant reverse transcriptase lacking the RNaseH activity was used. The results show that the RNaseH activity is needed to remove the 5' RNA sequences from the cDNA:RNA hybrid thereby enabling its translocation to another RNA containing an appropriate complementary target sequence.

PMID: 1711233 [PubMed - indexed for MEDLINE]

1: J Biol Chem 1995 Oct 13;270(41):24135-45 Related Articles, NEW **Books**, LinkOut

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Cleavage specificities of Moloney murine leukemia virus RNase H implicated in the second strand transfer during reverse transcription.

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Reverse transcription of a retroviral RNA genome requires two template jumps to generate the linear double-stranded DNA required for integration. The RNase H activity of reverse transcriptase has several roles during this process. We have examined RNase H cleavages that define the maximal 3' and 5' ends of Moloney murine leukemia virus minus strand DNA prior to the second template jump. In both the endogenous reaction and on model substrates in vitro, RNase H cleaves the genomic RNA template between the second and third ribonucleotides 5' of the U5/PBS junction, but other minor cleavages between 1 and 10 nucleotides 5' of this junction are also observed. Similar experiments examining the specificity of RNase H for tRNA primer removal revealed that cleavage generally leaves a ribo A residue at the 5' end of minus strand DNA. These observations suggest that three bases are typically duplicated on the ends of the minus strands, leading to an intermediate following the second jump which contains unpaired nucleotides. Model substrates mimicking the structure of this intermediate demonstrate that reverse transcriptase has little difficulty in utilizing such a branched structure for the initiation of displacement synthesis.

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